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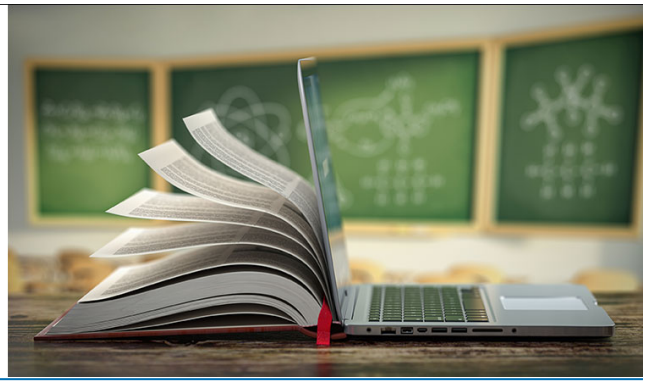

Prospects for photostimulation of nisin biosynthesis

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Abstract. The purpose of the article was to study the effect of blue spectrum light on the nisin producer Lactobacillus lactis. It was found that photostimulation of a Lactobacillus Lactis culture with blue spectrum light (435-470 nm) with a light flux intensity of 1800 mcd for 50-60 minutes positively affects the activity of nisin (increases by 60.1%), while the titer of the Lactobacillus culture lactis is 2 times higher. At the same time, there is a slight decrease in nisin titer after 24 hours of incubation, which is explained by the peculiarities of nisin biosynthesis: nisin is less active at the beginning of biosynthesis; during the period of the exponential growth phase, an increase in the biosynthesis of nisin is noted; the greatest activity of nisin is noted at the beginning of the stationary phase; nisin synthesis is reduced in the middle of the stationary phase of the cells; self-regulation of nisin synthesis (increased nisin synthesis leads to increased competition for metabolites of the substrate and energy material, nisin molecules act as an external factor that regulates synthesis). Light treatment of the nisin producer Lactobacillus lactis increases its resistance to oxidative stress and enhances its viability. As a result of studies during storage of the Lactobacillus lactis culture in skimmed milk, the positive effect of light on the high preservation of nisin activity was proved. Thus, photostimulation of the bioproducer of nisin - Lactobacillus Lactis culture with blue light with a light flux intensity of 1800 mcd for 50-60 minutes has a positive effect on its viability and allows us to recommend the use of blue light to increase nisin production.

1. Introduction

The increase in the duration of storage of food products is one of the promising areas of development of the food industry. A special place in ensuring the preservation of food products is given to the use of natural substances or beneficial microorganisms with a preservative effect, in particular bacteriocins. Many bacteriocins are known today, but the well-known and studied nisin is popular among food manufacturers. An important property that allows to use nisin in food industry is that it quickly separates in the human body into amino acids.

In this regard, it is advisable to characterize the nisin. Nisin GOST R 57646-2017 “Microbiological products. Food supplement nisin. Engineering specifications”, food supplement E 234– an antibiotic preparation of a polypeptide nature produced by lactic acid bacteria. Nisin has the form of a powder from white to light cream in color, soluble in water and aqueous solutions. The specimen is obtained by culturing the producing bacteria Lactococcuslactis and Streptococcuslactis in various natural substrates, in particular milk and whey, as well as glucose. Having a peptide structure, nisin is completely split in

the gastrointestinal tract under the influence of digestive enzymes and is absorbed. Acceptable daily intake (ADI) of nisin is 0.8 mg / kg body weight per day [1,2].

Nisin is actively used in the food industry. Thus, the addition of nisin to processed cheeses prevents the growth and development of spores of Clostridium sporogenes, Clostridium bityricum, Clostridium turobityricum and increases the shelf life by 2 times; in the composition of milk desserts nisin allows to increase the shelf life by 50%. Nisin is used as an inhibitor of starter cultures in yoghurts, which helps prevent their re-acidification. Nisin is added to liquid pasteurized soups and other canned foods, such as tomato marinade. Foreign manufacturers use nisin in sausages recipe. The production of nisin is carried out from strains of Lactobacillus lactis by fermentation. For the biosynthesis of nisin, a nutrient medium and liquid seed are preliminarily prepared, everything is placed in a fermenter and fermented, then micro and nanofiltration is carried out, the nanoconcentrate is salted out, dried and the compliance is confirmed [3].

The international unit for measuring the activity of antibiotics is ME - with 1 microgram of dry pure nisin corresponding to 40 ME.

To use it as a preservative dietary supplement is allowed by its properties:

- lack of toxicity;
- a natural metabolite of lactic acid bacteria;
- found in natural products (cheese, milk);
- bacteria that produce nisin are found in the human intestines;
- there is no cross-resistance to bacteria, which contributes to their tolerance to antibiotics used for therapeutic purposes;
- easily destroyed in the gastrointestinal tract, by enzymatic hydrolysis;
- hydrolysis of nisin already begins in the oral cavity under the action of saliva enzymes;
- it is not therapeutically applied;
- Nisin biosynthesis is carried out from bacterial strains that are safe for the body [4,5].

It should be noted that nisin is an inhibitor of bacterial spores that are resistant to thermal effects, which allows us to recommend it as an antimicrobial additive in meat technology. Nisin retains its antimicrobial properties at high pasteurization temperatures, the loss of nisin activity when it is autoclaved at a temperature of 120 ℃ is less than 10%, it dissolves well in a solution of 0.02 N hydrochloric acid. Moreover, in the presence of proteins, the ability to dissolve it increases as a result of adsorption by protein molecules, which is important in the production of meat products [1-4].

It should be noted that nisin can be used with other preservative substances.

It is advisable to consider the mechanism of the bactericidal action of nisin in order to justify the introduction of a biodegradable spathella into the formulation. Nisin belongs to the first class of bacteriocins or lantibiotics type “A”. Nisin acts on almost all gram-positive bacteria, in particular Streptoccus, Lactobacillus. At the same time, gram-negative microorganisms are mainly resistant to nisin, since their cell wall prevents the penetration of molecules of more than 700 Da, and in nisin the length of the molecule is 3353 Da, moreover, it is capable of polymerization and the formation of monomers up to 6700 Da. It is possible to enhance the penetration of nisin into the microbial cell by exposure to chelating compounds. The bactericidal effect of nisin is to destroy the cytoplasmic membrane of the microbial cell, which leads to the release of intracellular fluid, and, accordingly, the death of organelles and cell lysis. Известно несколько механизмов действия низина на мембрану клетки. Several mechanisms of the action of nisin on the cell membrane are known. For example, a nisin molecule is bound by phospholipids of the cell wall and cell peptidoglycans by electrostatic interaction, after which the membrane is compressed and the cell is destroyed. [7.8]

According to another mechanism, the action of nisin, the surface of the lipids of the cell bends and subsequently the formation of pores in the membrane occurs, which leads to the destruction of the microbial cell.

At the same time, there are mechanisms of resistance of microbial cells to nisin:

- thickening of the cell membrane;
- increase in pH on the surface of the cell wall;
- a change in the expression of genes involved in elongation of phospholipids, since the greater the compaction of phospholipids, the faster the penetration of nisin into the cell;
- release of antibiotics from the cell using MDR and ABC transporters [9-12].

These substances are able to bind compounds toxic to the cell and excrete them through the cell membrane [11].

Today, it seems relevant to increase the activity of nisin in order to reduce it in the formulation of food products.

It is known that visible light influences a microbial cell, depending on the spectral range it can enhance metabolic processes, respectively, the growth and reproduction rate, which is defined by the term “photostimulation”.

In this regard, the purpose of this study is to study the effect of blue spectrum light on the nisin producer Lactobacillus lactis.

2. Material and methods

For the experiment and biosynthesis of nisin, a one-day culture was used - the producer of nisin Lactobacillus lactis. Storage and maintenance of the culture was carried out by sowing in an agasized medium, incubated at a temperature of 30 ± 2 ℃ for 48 hours, and then spent a monthly passage on skimmed milk. The culture of Lactobacillus lactis was stored at a temperature of 4 to 6 ℃ in a refrigerator.

Culture samples of the Lactobacillus lactis control group were not treated before light inoculation. Samples of the experimental group of the producer of nisin before inoculation for 50-60 minutes were treated with an Avers-San biolamp (manufactured by NPK Avers CJSC, Moscow) with 20 LEDs built in with a wavelength of 435-470 nm and a radiation intensity of 1800 mcd.

A one-day producer culture in an amount of 100 ml was introduced into the fermentation medium (skimmed milk powder in an amount of 40 g, whey 400 ml, protolytic enzyme, 5 g of glucose and distilled water up to 1000 ml). Fermentation was carried out for 24 hours using a Biiphno laboratory Minipro-Lab fermenter at pH 6.8, the level of which was maintained by adding a 10% NaOH solution. Hydrolysis was carried out for two hours at a temperature of 28-30 ℃, sterilization for 30 minutes. Five fermentations of each group were done (n = 5).

Nisin activity was determined by a standard curve based on a standard nisin solution (preparation of a nisin solution in a 0.02 N hydrochloric acid solution with a nisin content of 5, 10, 20, 40 IU / ml). To determine the activity of nisin, 0.1 ml of standard and test solutions were added to the wells, thermostated at 55 ℃ for 16-18 hours, then the diameter of the growth inhibition zones of the Bacilus coagulans test culture was measured.

The titer of Lactobacillus lactis cells were determined by inoculation on an agassed medium consisting of meat peptone broth, yeast autolysate, minerals, enzymatic hydrolyzate of fish meal, bacteriological agar, glucose and sodium chloride.

To study the activity of Lactobacillus lactis culture cells during maintenance and storage, M 17 nutrient medium (made in Germany) was used, consisting of soy peptone, meat peptone, casein peptone, yeast extract, lactose, ascorbic acid, magnesium sulfate, sodium hydroxide solution, and distilled water.

The reaction was recorded after 6, 12 and 24 hours of incubation.The resistance of the culture of Lactobacillus lactis to oxidative stress was determined by inoculation it on a nutrient medium with 5 mM hydrogen peroxide in Petri dishes and incubating at a temperature of 30-32 ℃ for 48 hours. Sensitivity was investigated by the number of colonies grown. The reaction was recorded after 6, 12 and 24 hours of incubation.

3. Research results

We have studied the fermentation properties of samples of the control and experimental culture of Lactobacillus Lactis.

Figure 1 shows the activity of nisin during growth. From figure 1 it follows that the activity of nisin in the culture samples of the producer of the experimental group after 3, 6, 12.18 and 24 hours of growth is 1463, 4684, 6259, 9365 and 9246 IU / ml, which is 16.7% higher than the control, 17 5%, 46.3%, 61.9% and 60.1%. It should be noted that on the 24th day there is a decrease in the activity of nisin in the control and experimental groups.

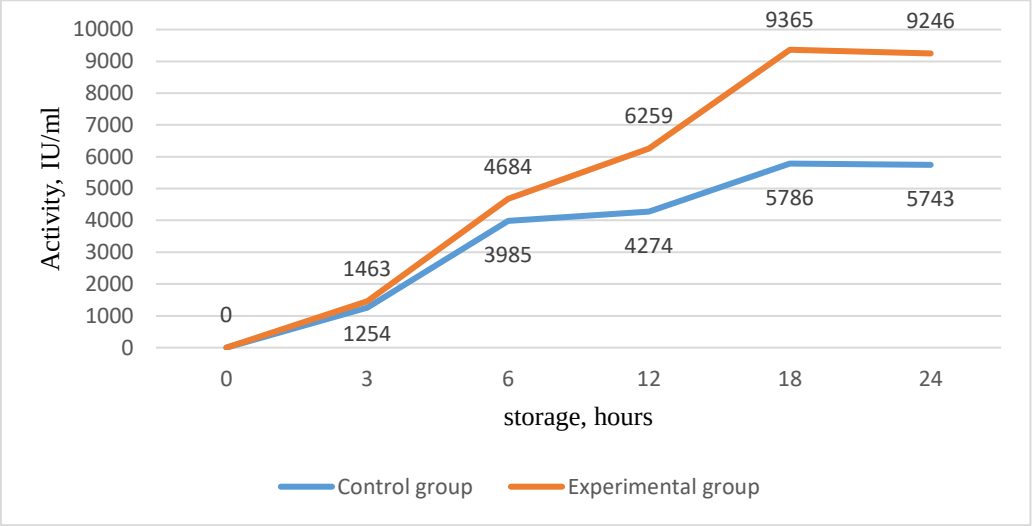

Figure 1. Nisin activity of the control and experimental culture of Lactobacillus lactis.

Figure 2 shows the titer of cultures of the control and experimental groups.

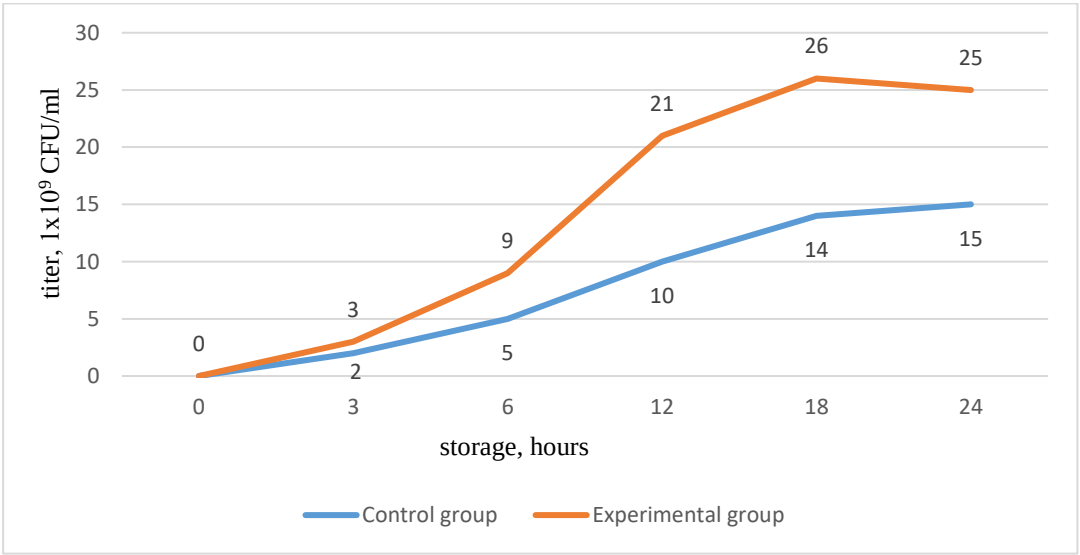

Figure 2. Titer of culture Lactobacillus lactis of control and experimental groups.

From figure 2 it follows that the titer of the culture of Lactobacillus lactis of the experimental group is significantly higher than in the control. So, the titer in the experimental group after 3, 6, 12, 18 and

24 hours of growth is 3, 9, 21, 26 and 25 x 109 CFU / ml, in the control - 2, 5, 10, 14 and 15 x 10 9 CFU / ml, respectively.

It follows from the results of the study that irradiating the Lactobacillus lactis culture with blue spectrum light (435-470 nm) with a light flux intensity of 1800 mcd for 50-60 minutes enhances the activity of nisin and increases the titer. In this regard, before the introduction of the films into the formulation, the lowlands were irradiated with visible light of the above spectrum for 50-60 minutes.

Studies on the resistance of the culture to oxidative stress, i.e. the effect of hydrogen peroxide on their growth. For the experiment, a one-day culture of Lactobacillus lactis was placed on agarized medium in Petri dishes (group 1); a culture of Lactobacillus lactis was placed on an agassed medium with 5 mM hydrogen peroxide in Petri dishes (group 2); a blue-irradiated culture of Lactobacillus lactis was placed on an agglomerated medium in Petri dishes (group 3) and a blue-irradiated culture of Lactobacillus lactis was placed on an agglomerated medium with 5 mM hydrogen peroxide in Petri dishes (group 4). Incubated at a temperature of 30-32 ℃ for 48 hours, the sensitivity was investigated by the number of grown colonies. The reaction was recorded after 6, 12 and 24 hours of incubation.

Table 1 shows the effect of hydrogen peroxide on the growth of Lactobacillus lactis.

Table 1. The effect of hydrogen peroxide on the growth of Lactobacillus lactis, x 10^3 CFU / ml.

Growth hours	Group			
	1	2	3	4
6	4	1	9	3
12	9	3	17	6
24	14	5	23	8

From the data of table 1 it follows that oxidative stress affects the viability of the culture, both treated with blue light (group 4), and native (group 2). The titer of the culture of Lactobacillus lactis incubated in a medium without hydrogen peroxide (group 1) after 6, 12 and 24 hours is 4, 9 and 14 x 10^3 CFU/ml, while the culture grown in a nutrient medium with hydrogen peroxide is 1, 3 and 5 x 10^3 CFU/ml. As noted earlier, treatment of the Lactobacillus lactis culture with blue spectrum light positively affects the growth of microorganisms, but oxidative stress leads to a decrease in titer. So, the amount of Lactobacillus lactis in the third group after 6, 12 and 24 incubations at the level of 9, 17 and 23 x 103 CFU / ml, in the 4th group - 3, 6 and 8 x 103 CFU / ml.

Studies of the activity of nisin during storage of the Lactobacillus lactis culture in skim milk for three days at a temperature of 4 to 6 ℃ in the refrigerator were performed.

Table 2 presents the results of a study of the activity of nisin during storage of a Lactobacillus lactis culture (group 1 - native culture, group 2 — culture samples treated with blue light) stored in skimmed milk.

Table 2. Nisin Activity from Lactobacillus Lactis Culture Samples in skimmed milk, %.

Group 1				Group 2			
Duration of storage, months							
background	1	2	3	background	1	2	3
100	100	92	85	100	100	94	92

From the data of table 2 it is seen that the activity of nisin in the samples of cultures treated with light of the blue spectrum during storage is higher. So, after 1, 2 and 3 months of storage is 100%, 94% and 92%, in the first group - 100%, 92% and 85%, respectively.

The results obtained indicate a positive effect of blue spectrum light on the viability of Lactobacillus lactis cells during storage.

4. Conclusion

From the results of the studies it follows that photostimulation of a Lactobacillus Lactis culture with blue spectrum light (435-470 nm) with a light flux intensity of 1800 mcd for 50-60 minutes positively affects the activity of nisin (increases by 60.1%), while the culture titer Lactobacillus lactis is 2 times higher. At the same time, a slight decrease in the nisin titer after 24 hours of incubation was observed, which is explained by the peculiarities of nisin biosynthesis: nisin is less active at the beginning of biosynthesis; during the period of the exponential growth phase, an increase in the biosynthesis of nisin is noted; the greatest activity of nisin is noted at the beginning of the stationary phase; nisin synthesis is reduced in the middle of the stationary phase of the cells; self-regulation of nisin synthesis (increased nisin synthesis leads to increased competition for metabolites of the substrate and energy material, nisin molecules act as an external factor that regulates synthesis). Light treatment of the nisin producer Lactobacillus lactis increases its resistance to oxidative stress and enhances its viability. As a result of studies during storage of the Lactobacillus lactis culture in skim milk, the positive effect of light on the high preservation of nisin activity was proved.

Therefore, photostimulation of the nisin bioproducer - Lactobacillus Lactis culture with blue light with a light flux intensity of 1800 mcd for 50-60 minutes positively affects its viability and allows us to recommend the use of blue light to increase nisin production.

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